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(57) Abstract

Tissue cells of an animal transformed with a recombinant vector construct which (a) directs the expression of a protein or active portion thereof; (b) transcribes an antisense message; or (c) transcribes a ribozyme capable of inhibiting MHC antigen presentation are provided. In a related aspect, the cells are transformed with two or more of such proteins, antisense or ribozymes, or combinations thereof. The tissue cells are particularly useful within methods for suppressing graft rejection. Pharmaceutical compositions comprising such transformed tissue cells are also provided.

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Description

METHODS OF SUPPRESSING GRAFT REJECTION

Technical Field

The present invention relates generally to the field of tissue transplantation, and more specifically, to methods for preventing graft rejection mediated by T-cell recognition and activation.

Background of the Invention

Effective and safe methods of suppressing the immune response have been a critical issue for clinical transplants from the beginning of the 1950's. Upon introduction of allograft tissue into an animal, an attack by the immune system is initiated consisting of both humoral and cell mediated responses. In these responses, tissue cells are targeted for clearance by antibodies directed against the transplanted tissues or destroyed by killer cells. Allograft rejection consists of a series of complex T-cell dependent events triggered by donor histocompatibility molecules. One major event in T-cell activation is associated with the *de novo* expression of the cell surface protein, interleukin-2 receptor (IL-2R) which is essential for proliferation and continued viability of alloactivated T-cells upon binding to IL-2. (Cantrell et al., Science 224:1312, 1984). When IL-2R is not present, the immune response is suppressed.

One early attempt to suppress the rejection response utilized whole-body irradiation. However, such attempts were unsuccessful due to a lack of specificity and increased tumor production. This led to the utilization of either specific drugs or antibodies for the prevention and treatment of graft rejection. In this regard, a number of drugs (Carpenter et al., New Engl. J. Med. 32:1224, 1991) have been shown to be successfully immunosuppressive, but not without various side effects. These toxic effects generally include neurologic, dermal, gastrointestinal, endocrine, vascular, and hematologic complications. One such established drug that induces specific tolerance to organ transplants is cyclosporine. This drug exerts its therapeutic affect by inhibiting T-cell-mediated alloimmune and autoimmune responses specifically by suppression of IL-2 production at the mRNA transcriptional level (Kronke et al., PNAS 81:5214, 1984). Although the exact mechanism is not yet known, reduction of IL-2 synthesis has been demonstrated *in vivo* in bone marrow transplants (Hess et al., J. Immunol. 128:355, 1983) and renal-transplant recipients (Azogui et al., J. Immunol. 131:1205,

1983) by repetitive drug administration. However, since cyclosporine is non-specifically administered throughout the systemic circulation, the drug is known to have many toxic side-effects, for example hepatotoxicity and nephrotoxicity (Kahan et al., New Engl. J. Med. 321:1725, 1989). In addition, administration of cyclosporine renders the patient more susceptible to general infection.

Antibodies directed to the lymphoid cells of the immune system have also been used in anti-rejection therapy, starting in the 1960's (Filo et al., Transplantation 30:445, 1980). However, such anti-lymphocyte globulins, although generally useful, were of variable potency and had the potential disadvantage of containing antibodies directed against a wide variety of nonlymphoid tissues, such as platelets and macrophages. The first clinical antibody to be used was anti-CD3, also known as OKT3. OKT3 is directed only against mature T-lymphocytes, its precise target being the CD3 cluster that composes the antigen-receptor complex of T-cells. The $F(ab)_2$ fragment of the OKT3 monoclonal antibody retains the immunosuppressive properties of the whole antibody but is less active in eliciting T-cell activation and lymphokine release (Woodle et al., Transplantation 52:354, 1991). Bioengineered variants of the OKT3 molecule with high epitope specificity and high immune suppression potency have been produced. However, the antibody has the disadvantage of activating all accessible T-cells, sometimes resulting in severe febrile and circulatory problems for the first day or two after administration (Carpenter, Am. J. Kidney Dis. 14:suppl 2:1, 1989). Monoclonal antibodies directed at surface receptors other than CD3 have yielded mixed results (Heffron et al., Transplant Sci. 1:64, 1991). Although anti-CD4 monoclonal antibodies have appeared more attractive in view of their low toxicity and propensity to induce longer-lasting immunologic non-responsiveness in certain animal models, the depletion of $CD4^+$ T-cells may lead to a possible AIDS syndrome (Sablinski et al., Transplantation 52:579, 1991). Therefore, these systems do not provide suitable long-term effects and must be repetitively administered.

Consequently, there is a need in the art for improved methods of suppressing the immune response, without the side effects or disadvantages of previously described methods. The present invention fulfills these needs and further provides other related advantages.

Summary of the Invention

Briefly stated, the present invention provides methods for suppressing MHC antigen presentation in order to suppress the immune response of T-cells, including cytotoxic T-lymphocytes (CTL), thereby preventing graft rejection. Within

one aspect, a method is provided for suppressing graft rejection, comprising transforming tissue cells isolated from a donor animal with a recombinant vector construct which directs the expression of a protein or active portion of a protein capable of inhibiting MHC antigen presentation, and transplanting the transformed tissue cells into a recipient animal such that an immune response against the tissue cells is suppressed. Within one embodiment of the invention, the recombinant vector construct directs the expression of a protein capable of binding β_2-microglobulin, such as H301. With another embodiment, the recombinant vector construct directs the expression of a protein capable of binding the MHC class I heavy chain molecule intracellularly, such as E3/19K.

Within another aspect of the invention, a method is provided for suppressing graft rejection, comprising transforming tissue cells isolated from a donor animal with a recombinant vector construct which transcribes an antisense message, the antisense message being capable of inhibiting MHC antigen presentation, and transplanting the transformed tissue cells into a recipient animal such that an immune response against the tissue cells is suppressed. Within certain embodiments of the present invention, the recombinant vector construct transcribes an antisense message which binds to a conserved region of the MHC class I heavy chain transcripts, β_2-microglobulin transcript, or the PSF1 transporter protein transcript.

Within still another aspect of the invention, a method is provided for suppressing graft rejection, comprising transforming tissue cells isolated from a donor animal with a recombinant vector construct which transcribes a ribozyme capable of inhibiting MHC antigen presentation, and transplanting the transformed tissue cells into a recipient animal such that an immune response against the tissue cells is suppressed. Within certain embodiments of the invention, the recombinant vector construct transcribes a ribozyme that cleaves a conserved region of MHC class I heavy chain transcripts, β_2-microglobulin transcript or the PSF1 transporter protein transcript.

Within another aspect of the invention, a method is provided for suppressing graft rejection comprising transforming tissue cells isolated from a donor animal with a multivalent recombinant vector construct which directs the expression of a protein or active portion of a protein capable of inhibiting MHC antigen presentation, and an antisense or ribozyme capable of inhibiting MHC antigen presentation, and transplanting the transformed tissue cells into a recipient animal such that an immune response against the tissue cells is suppressed. Within a related aspect, tissue cells isolated from a donor animal are transformed with a multivalent recombinant vector construct which directs the expression of an antisense message and a ribozyme capable

of inhibiting MHC antigen presentation. Subsequently, the transformed tissue cells are transplanted into a recipient animal such that an immune response against the tissue cells is suppressed. Within another related aspect of the invention, tissue cells isolated from a donor animal are transformed with a multivalent recombinant vector construct which directs the expression of two or more proteins or active portions of proteins capable of inhibiting MHC antigen presentation, two or more antisense messages capable of inhibiting MHC antigen presentation, or two or more ribozymes capable of inhibiting MHC antigen presentation. Subsequently, the transformed tissue cells are transplanted into a recipient animal such that an immune response against the tissue cells is suppressed.

Within various embodiments of the invention, the multivalent recombinant vector construct expresses or transcribes at least two of the following in any combination: a protein or active portion of a protein selected from the group consisting of E3/19K and H301, an antisense message that binds to the transcript of a conserved region of MHC class I heavy chains, β_2-microglobulin or PSF1 transporter protein, or a ribozyme that cleaves the transcript of a conserved region of MHC class I heavy chains, β_2-microglobulin or PSF1 transporter protein. Within another embodiment, the multivalent recombinant viral vector constructs express or transcribe two such proteins or active portions of the proteins, two antisense messages or two ribozymes.

Within preferred embodiments, the recombinant vector construct is a recombinant viral vector construct. Within a particularly preferred embodiment, the recombinant vector construct is a recombinant retroviral vector construct. Within other embodiments, the recombinant viral vector construct is carried by a recombinant virus selected from the group consisting of togaviridae, picornaviridae, poxviridae, adenoviridae, parvoviridae, herpesviridae, paramyxoviridae and coronaviridae viruses.

In the context of the present invention, suitable donor tissue cells include bone marrow cells, pancreatic islet cells, fibroblast cells, corneal cells and skin cells. Such tissue cells may be transplanted into a recipient animal using a number of methods, including direct injection or catheter infusion.

Within still another related aspect of the present invention, pharmaceutical compositions are is provided comprising tissue cells transformed with a recombinant vector construct or a multivalent recombinant vector construct as described herein.

Within various embodiments of the present invention methods are provided wherein the transformed tissue cells are implanted into an animal having the

same type MHC, into a different animal species from which the tissue cells were removed.

These and other aspects of the present invention will become evident upon reference to the following detailed description.

Detailed Description of the Invention

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

"Transplant" refers to the insertion or grafting of tissue cells into a recipient animal such that at least a portion of the tissue cells are viable subsequent to implantation. The implanted tissue can be placed within tissue of similar function or of different function. For example, tissue cells from one animal may be removed and transformed with recombinant vector constructs before being "implanted" into another animal. Transplantation of tissue between genetically dissimilar animals of the same species is termed allogeneic transplantation.

"Transforming" tissue cells refers to the transduction or transfection of tissue cells by any of a variety of means recognized by those skilled in the art, such that the transformed tissue cell expresses additional polynucleotides as compared to a tissue cell prior to the transforming event.

"Recombinant vector construct" or "vector construct" refers to an assembly which is capable of expressing sequences or genes of interest. In the context of protein expression, the vector construct must include promoter elements and may include a signal that directs polyadenylation. In addition, the vector construct preferably includes a sequence which, when transcribed, is operably linked to the sequences or genes of interest and acts as a translation initiation sequence. Preferably, the vector construct includes a selectable marker such as neomycin, thymidine kinase, hygromycin, phleomycin, histidinol, or dihydrofolate reductase (DHFR), as well as one or more restriction sites and a translation termination sequence. In addition, if the vector construct is used to make a retroviral particle, the vector construct must include a retroviral packaging signal and LTRs appropriate to the retrovirus used, provided these are not used already present. The vector construct can also be used in combination with other viral vectors or inserted physically into cells or tissues as described below. As noted above, the vector construct includes a sequence that encodes a protein or active portion of the protein, antisense or ribozyme. Such sequences are designed to inhibit MHC antigen presentation in order to suppress the immune response of cytotoxic T-lymphocytes against the transplanted tissue.

In general, the recombinant vector constructs described herein are prepared by selecting a plasmid with a strong promoter, and appropriate restriction sites for insertion of DNA sequences of interest downstream from the promoter. As noted above, the vector construct may have a gene encoding antibiotic resistance for selection as well as termination and polyadenylation signals. Additional elements may include enhancers and introns with functional splice donor and acceptor sites.

The construction of multivalent recombinant vector constructs may require two promoters when two proteins are being expressed, because one promoter may not ensure adequate levels of gene expression of the second gene. In particular, where the vector construct expresses an antisense message or ribozyme, a second promoter may not be necessary. Within certain embodiments, an internal ribosome binding site (IRBS) or herpes simplex virus thymidine kinase (HSVTK) promoter is placed in conjunction with the second gene of interest in order to boost the levels of gene expression of the second gene. Briefly, with respect to IRBS, the upstream untranslated region of the immunoglobulin heavy chain binding protein has been shown to support the internal engagement of a bicistronic message (Jacejak et al., Nature 353:90, 1991). This sequence is small, approximately 300 base pairs, and may readily be incorporated into a vector in order to express multiple genes from a multi-cistronic message whose cistrons begin with this sequence.

Where the recombinant vector construct is carried by a virus, such constructs are prepared by inserting sequences of a virus containing the promoter, splicing, and polyadenylation signals into plasmids containing the desired gene of interest using methods well known in the art. The recombinant viral vector containing the gene of interest can replicate to high copy number after transduction into the target tissue cells.

Subsequent to preparation of the recombinant vector construct, it may be preferable to assess the ability of vector transformed cells to down regulate MHC antigen presentation. In general, such assessments may be performed by Western blot, FACS analysis, or by other methods recognized by those skilled in the art.

Within preferred embodiments, the recombinant vector construct is carried by a retrovirus. Retroviruses are RNA viruses with a single positive strand genome which in general, are nonlytic. Upon infection, the retrovirus reverse transcribes its RNA into DNA, forming a provirus which is inserted into the host cell genome. Preparation of retroviral constructs for use in the present invention is described in greater detail in an application entitled "Recombinant Retroviruses" (U.S.S.N. 07/586,603, filed September 21, 1990) herein incorporated by reference. The

retroviral genome can be divided conceptually into two parts. The "trans-acting" portion consists of the region coding for viral structural proteins, including the group specific antigen (gag) gene for synthesis of the core coat proteins; the pol gene for the synthesis of the reverse transcriptase and integrase enzymes; and the envelope (env) gene for the synthesis of envelope glycoproteins. The "cis-acting" portion consists of regions of the genome that is finally packaged into the viral particle. These regions include the packaging signal, long terminal repeats (LTR) with promoters and polyadenylation sites, and two start sites for DNA replication. The internal or "trans-acting" part of the cloned provirus is replaced by the gene of interest to create a "vector construct". When the vector construct is placed into a cell where viral packaging proteins are present (see U.S.S.N. 07/800,921), the transcribed RNA will be packaged as a viral particle which , in turn, will bud off from the cell. These particles are used to transduce tissue cells, allowing the vector construct to integrate into the cell genome. Although the vector construct express its gene product, the virus carrying it is replication defective because the trans-acting portion of the viral genome is absent. Various assays may be utilized in order to detect the presence of any replication competent infectious retrovirus. One preferred assay is the extended S^+L^- assay described in Example 9. Preferred retoviral vectors include murine leukemia amphotropic or xenotropic, or VsVg pseudotype vectors (see WO 92/14829; and U.S.S.N. 08/____ to be assigned by PTO incorporated herein by reference).

Recombinant vector constructs may also be developed and utilized with a variety of viral carriers including, for example, poliovirus (Evans et al., Nature 339:385, 1989, and Sabin et al., J. of Biol. Standardization 1:115, 1973) (ATCC VR-58); rhinovirus (Arnold et al., J. Cell. Biochem. L401, 1990) (ATCC VR-1110); pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch et al., PNAS 86:317, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86, 1989; Flexner et al., Vaccine 8:17, 1990; U.S. 4,603,112 and U.S. 4,769,330; WO 89/01973) (ATCC VR-111; ATCC VR-2010); SV40 (Mulligan et al., Nature 277:108, 1979) (ATCC VR-305), (Madzak et al., J. Gen. Vir. 73:1533, 1992); influenza virus (Luytjes et al., Cell 59:1107, 1989; McMicheal et al., The New England Journal of Medicine 309:13, 1983; and Yap et al., Nature 273:238, 1978) (ATCC VR-797); adenovirus (Berkner et al., Biotechniques 6:616, 1988, and Rosenfeld et al., Science 252:431, 1991) (ATCC VR-1); parvovirus such as adeno-associated virus (Samulski et al., J. Vir. 63:3822, 1989, and Mendelson et al., Virology 166:154, 1988) (ATCC VR-645); herpes simplex virus (Kit et al., Adv. Exp. Med. Biol. 215:219, 1989) (ATCC VR-977; ATCC VR-260); Nature 277: 108, 1979); HIV (EPO 386,882, Buchschacher et al., J. Vir. 66:2731, 1992); measles virus

(EPO 440,219) (ATCC VR-24); Sindbis virus (Xiong et al., Science 234:1188, 1989) (ATCC VR-68); and coronavirus (Hamre et al., Proc. Soc. Exp. Biol. Med. 121:190, 1966) (ATCC VR-740). It will be evident to those in the art that the viral carriers noted above may need to be modified to express proteins, antisense messages or ribozymes capable of inhibiting MHC antigen presentation.

Once a vector construct has been prepared, it may be used to transform isolated tissue cells through a variety of routes. More specifically, naked DNA or a recombinant viral vector construct containing a gene that codes for a protein or active portion of a protein, an antisense message or ribozyme capable of inhibiting MHC antigen presentation, may be introduced into tissue cells removed from a donor using physical methods or through the use of viral or retroviral vectors as discussed herein.

Ex vivo procedures for physical and chemical methods of uptake include calcium phosphate precipitation, direct microinjection of DNA into intact target cells, and electroporation whereby cells suspended in a conducting solution are subjected to an intense electric field in order to transiently polarize the membrane, allowing entry of macromolecules. Other procedures include the use of DNA bound to ligand, DNA linked to an inactive adenovirus (Cotton et al., PNAS 89: 6094, 1990), bombardment with DNA bound to particles, liposomes entrapping recombinant vector construct, spheroplast fusion whereby *E. coli* containing recombinant viral vector constructs are stripped of their outer cell walls and fused to animal cells using polyethylene glycol and viral transduction, (Cline et al., Pharmac. Ther. 29:69, 1985; and Friedmann et al., Science 244:1275, 1989). Alternatively, as noted above, the vector construct may be carried by a virus such as vaccinia, Sindbis or corona virus. Further, methods for administering a vector construct via a retroviral vector are described in more detail in an application entitled "Recombinant Retroviruses" (U.S.S.N. 07/586,603) herein incorporated by reference.

In an *ex vivo* context, the transformed cells are transplanted into the animal, and monitored for gene expression as described in Examples 15. Protocols vary depending on the tissue cells chosen. Briefly, a recombinant vector construct carrying a sequence, the expression of which inhibits MHC class I presentation, is transformed into tissue cells. Preferable 10^5 to 10^9 tissue cells are transformed. The cells are cultured, and transformed cells may be selected by antibiotic resistance. Cells are assayed for gene expression by Western blot and FACS analysis, or other means. For example, as described in more detail below, bone marrow cells that have been transformed are transplanted in an animal by intravenous administration of 2 to 3 x 10^7 cells (see WO 93/00051).

Cells that can be transformed include, but are not limited to, fibroblast cells, bone marrow cells, endothelial cells, keratinocytes, hepatocytes, and thyroid follicular cells. Transformed cells may be administered to patients directly by intramuscular, intradermal, subdermal, intravenous, or direct catheter infusion into cavities of the body. *In vivo* gene expression of transduced bone marrow cells is detected by monitoring hematopoesis as a function of hematocrit and lymphocyte production.

It will be evident to those skilled in the art that isolated pancreatic islet cells can also be transformed as described above. Such transformed cells may then be transplanted into recipients by injection through the gastro-epiploic artery. *In vivo* gene expression of insulin is observed by monitoring blood glucose levels.

As discussed above, the present invention provides methods and compositions suitable for inhibiting MHC antigen presentation in order to suppress the immune response of the host. Briefly, CTL are specifically activated by the display of processed peptides in the context of self MHC molecules along with accessory molecules such as CD8, intercellular adhesion molecule -1 (ICAM-1), ICAM-2, ICAM-3, leukocyte functional antigen-1 (LFA-1) (Altmann et al., Nature 338:521, 1989), the B7/BB1 molecule (Freeman et al., J. Immunol. 143:2714, 1989), LFA-3 (Singer, Science 255:1671, 1992; Rao, Crit. Rev. Immunol. 10:495, 1991), or other cell adhesion molecules. Antigenic peptide presentation in association with MHC class I molecules leads to CTL activation. Transfer and stable integration of specific sequences capable of expressing products expected to inhibit MHC antigen presentation block activation of T-cells, such as $CD8^+$ CTL, and therefore suppress graft rejection. A standard CTL assay is used to detect this response as described in more detail in Example 13. Components of the antigen presentation pathway include the 45Kd MHC class I heavy chain, β_2-microglobulin, processing enzymes such as proteases, accessory molecules, chaperones, and transporter proteins such as PSF1.

Within one aspect of the present invention, vector constructs are provided which direct the expression of a protein or active portion of a protein capable of inhibiting MHC class I antigen presentation. Within the present invention, an "active portion" of a protein is that fragment of the protein which must be retained for biological activity. Such fragments or active domains can be readily identified by systematically removing nucleotide sequences from the protein sequence, transforming target cells with the resulting recombinant vector construct, and determining MHC class I presentation on the surface of cells using FACS analysis or other immunological assays, such as a CTL assay. These fragments are particularly useful when the size of

the sequence encoding the entire protein exceeds the capacity of the viral carrier. Alternatively, the active domain of the MHC antigen presentation inhibitor protein can be enzymatically digested and the active portion purified by biochemical methods. For example, a monoclonal antibody that blocks the active portion of the protein can be used to isolate and purify the active portion of the cleaved protein (Harlow et al., Antibodies: A Laboratory Manual, Cold Springs Harbor, 1988).

Within one embodiment, the recombinant vector construct directs the expression of a protein or active portion of a protein that binds to newly synthesized MHC class I molecules intracellularly. This binding prevents migration of the MHC class I molecule from the endoplasmic reticulum, resulting in the inhibition of terminal glycosylation. This blocks transport of these molecules to the cell surface and prevents cell recognition and lysis by CTL. For instance, one of the products of the E3 gene may be used to inhibit transport of MHC class I molecules to the surface of the transformed cell. More specifically, E3 encodes a 19kD transmembrane glycoprotein, E3/19K, transcribed from the E3 region of the adenovirus 2 genome. Within the context of the present invention, tissue cells are transformed with a recombinant vector construct containing the E3/19K sequence, which upon expression produces the E3/19K protein. The E3/19K protein inhibits the surface expression of MHC class I surface molecules, and cells transformed by the vector construct evade an immune response. The construction of a representative recombinant vector construct in this regard is presented in Example 2. Consequently, donor cells can be transplanted with reduced risk of graft rejection and may require only a minimal immunosupressive regimen for the transplant patient. This allows an acceptable donor-recipient chimeric state to exist with fewer complications.

Within another embodiment of the present invention, the recombinant vector construct directs the expression of a protein or an active portion of a protein capable of binding β_2-microglobulin. Transport of MHC class I molecules to the cell surface for antigen presentation requires association with β_2-microglobulin. Thus, proteins that bind β_2-microglobulin and inhibit its association with MHC class I indirectly inhibit MHC class I antigen presentation. Suitable proteins include the H301 gene product. Briefly, the H301 gene, obtained from the human cytomegalovirus (CMV) encodes a glycoprotein with sequence homology to the β_2-microglobulin binding site on the heavy chain of the MHC class I molecule (Browne et al., Nature 347:770, 1990). H301 binds β_2-microglobulin, thereby preventing the maturation of MHC class I molecules, and renders transformed cells unrecognizable by cytotoxic T-cells, thus evading MHC class I restricted immune surveillance.

Other proteins, not discussed above, that function to inhibit or down-regulate MHC class I antigen presentation may also be identified and utilized within the context of the present invention. In order to identify such proteins, in particular those derived from mammalian pathogens (and, in turn, active portions thereof), a recombinant vector construct that expresses a protein or an active portion thereof suspected of being capable of inhibiting MHC class I antigen presentation is transformed into a tester cell line, such as BC. The tester cell lines with and without the sequence encoding the candidate protein are compared to stimulators and/or targets in the CTL assay. A decrease in cell lysis corresponding to the transformed tester cell indicates that the candidate protein is capable of inhibiting MHC presentation.

An alternative method to determine down-regulation of MHC class I surface expression is by FACS analysis. More specifically, cell lines are transformed with a recombinant vector construct encoding the candidate protein. After drug selection and expansion, the cells are analyzed by FACS for MHC class I expression and compared to that of non-transformed cells. A decrease in cell surface expression of MHC class I indicates that the candidate protein is capable of inhibiting MHC presentation (see, for instance, Example 12).

Within another aspect of the present invention, methods are provided for suppressing graft rejection by transforming tissue cells with a recombinant vector construct which transcribes an antisense message capable of inhibiting MHC class I antigen presentation. Briefly, oligonucleotides with nucleotide sequences complementary to the protein coding or "sense" sequence are termed "antisense". Antisense RNA sequences function as regulators of gene expression by hybridizing to complementary mRNA sequences and arresting translation (Mizuno et al., PNAS 81:1966, 1984; Heywood et al., Nucleic Acids Res. 14:6771, 1986). Antisense molecules comprising the entire sequence of the target transcript or any part thereof can be synthesized (Ferretti et al., PNAS 83:599, 1986), placed into vector constructs, and effectively introduced into cells to inhibit gene expression (Izant et al., Cell 36:1007, 1984). In addition, the synthesis of antisense RNA (asRNA) from DNA cloned in inverted orientation offers stability over time while constitutive asRNA expression does not interfere with normal cell function.

Within one embodiment of the present invention, the recombinant viral vector construct transcribes an antisense message capable of binding to a conserved region of the MHC class I transcripts, thereby inhibiting cell surface expression and MHC class I antigen presentation. One may identify such conserved regions through computer-assisted comparison of sequences representing different classes of MHC

genes (for example, HLA A, B and C), available within DNA sequence databanks (e.g., Genbank). Conserved sequences are then identified through computer-assisted alignment for homology of the nucleotide sequences. The conserved region is a sequence having less than 50% mismatch, preferably less than 20% mismatch, per 100 base pairs between MHC class I genotypes.

Within another embodiment of the present invention, the recombinant vector construct transcribes an antisense message responsible for binding to β_2-microglobulin transcript. This binding prevents translation of the β_2-microglobulin protein and thereby inhibits proper assembly of the MHC class I molecule complex necessary for cell surface expression. Within a preferred embodiment, the nucleotide sequence for β_2-microglobulin is cloned into a vector construct in the reverse orientation. The proper antisense orientation may be determined by restriction enzyme analysis.

Within still another embodiment of the present invention, the recombinant vector construct transcribes an antisense message responsible for binding PSF1 transcript, a peptide transporter protein. Since this protein is necessary for the efficient assembly of MHC class I molecules, such an antisense blocks the transport of processed antigenic peptide fragments to the endoplasmic reticulum (ER) prior to association with the MHC class I molecular complex. Within a preferred embodiment, the nucleotide sequence for the antisense PSF1 is prepared and inserted in reverse orientation into the vector construct and determined by restriction enzyme analysis.

As discussed above, the sequences of other proteins involved in antigen presentation may also be identified, and used to design a recombinant vector construct capable of transcribing an antisense RNA message that inhibits MHC antigen presentation. More specifically, the nucleotide sequence of the gene encoding the protein is examined, and the identified sequence is used to synthesize an appropriate antisense message. It is preferable to use a sequence complimentary to a portion upstream or close to the start sequence of the target message. This allows the antisense sequence to bind to the mRNA preventing translation of a significant portion of the protein. Examples of such molecules are ICAM-1, ICAM-2, ICAM-3, LFA-1, LFA-3, and B7/BB1. Down-regulation of MHC class I expression or antigen presentation may be assayed by FACS analysis or CTL assay, respectively, as described in Examples 14 and 15 or by other means as described above for proteins capable of inhibiting MHC class I presentation.

Within another aspect of the present invention, a method is provided for suppressing an immune response within an animal by transforming selected cells of the

animal with a recombinant vector construct which transcribes a ribozyme responsible for the enzymatic cleavage of a component involved in the MHC antigen presentation. Briefly, ribozymes are RNA molecules with enzymatic cleaving activity which are used to digest other RNA molecules. They consist of short RNA molecules possessing highly conserved sequence-specific cleavage domains flanked by regions which allow accurate positioning of the enzyme relative to the potential cleavage site in the desired target molecule. They provide highly flexible tools in inhibiting the expression and activation of specific genes (Haseloff et al., Nature 334:585, 1988). Custom ribozymes can easily be designed, provided that the transcribed sequences of the gene are known. Specifically, a ribozyme may be designed by first choosing the particular target RNA sequence and attaching complimentary sequences to the beginning and end of the ribozyme coding sequence. This ribozyme producing gene unit can then be inserted into a recombinant vector construct and used to transform tissue cells. Upon expression, the target gene is neutralized by complimentary binding and cleavage, guaranteeing permanent inactivation. In addition, because of their enzymatic activity, ribozymes are capable of destroying more than one target.

Within one embodiment of the present invention, recombinant vector construct containing specific ribozymes are used to cleave the transcript of a conserved region of the MHC class I molecule in order to inhibit antigen presentation. Within another embodiment of the present invention, the recombinant vector construct transcribes a ribozyme responsible for the enzymatic cleavage of the β_2-microglobulin transcript. Specifically, a ribozyme with flanking regions complimentary to a sequence of the β_2-microglobulin message cleaves the transcript, thereby preventing protein translation and proper assembly of the MHC class I molecule complex. This inhibits transport of the MHC class I complex to the cell surface, thereby suppressing antigen presentation.

Within still another embodiment of the present invention, the recombinant vector construct transcribes a ribozyme responsible for the enzymatic cleavage of the PSF1 transcript, thereby suppressing cell surface expression of MHC class I molecules and preventing antigen presentation. Specifically, a ribozyme designed with flanking regions complimentary to a sequence of the PSF1 message cleaves the transcripts and inhibits transport of peptides to the ER, thereby preventing assembly of the MHC class I complex and antigen presentation.

It will be evident to those skilled in the art that the sequences of other proteins involved in MHC antigen presentation (see above) can be identified and used to design a recombinant vector construct capable of transcribing a ribozyme that

inhibits MHC antigen presentation. Down regulation of MHC class I expression or antigen presentation may be assayed by CTL analysis, respectively, or other means as described above for proteins capable of inhibiting MHC class I presentation.

Within another aspect of the invention, multivalent recombinant vector constructs are provided. Briefly, the efficiency of suppressing an autoimmune response can be enhanced by transforming cells with a multivalent recombinant vector construct. Upon expression, the gene products increase the degree of interference with MHC antigen presentation by attacking a single component via two different routes or two different components via the same or different routes. The construction of multivalent recombinant vector constructs may require two promoters because one promoter may not ensure adequate levels of gene expression of the second gene. A second promoter, such as an internal ribzome binding site (IRBS) promoter, or herpes simplex virus thymidine kinase (HSVTK) promoter placed in conjunction with the second gene of interest boosts the levels of gene expression of the second gene.

Within preferred embodiments, the vector construct expresses or transcribes at least two of the following components in any combination: (a) a protein or active portion of the proteins E3/19K or H301; (b) an antisense message that binds the transcript of a conserved region of the MHC class I heavy chain, β_2-microglobulin or PSF1 transporter protein; and (c) a ribozyme that cleaves the transcript of the proteins listed in (b) above. In addition, multivalent recombinant vector constructs are provided which express two proteins or active portions of proteins as described herein, two antisense messages, or two ribozymes.

Within related embodiments, a number of specific combinations may be utilized to form a multivalent recombinant vector construct. For example, a multivalent recombinant vector construct may consist of a gene expressing E3/19K or H301 in combination with the antisense or ribozyme message for a conserved region of the MHC class I heavy chain, β_2-microglobulin, or PSF1 transporter protein.

Within another aspect of the present invention, pharmaceutical compositions are provided comprising one of the above described recombinant vector constructs or a recombinant virus carrying the vector construct, such as a retrovirus, poliovirus, rhinovirus, vaccinia virus, influenza virus, adenovirus, adeno-associated virus, herpes simplex virus, SV40, HIV, measles virus, coronavirus or Sindbis virus, in combination with a carrier or diluent. The composition may be prepared either as a liquid solution, or as a solid form (e.g., lyophilized) which is suspended in a solution prior to transforming tissue cells *ex vivo*.

In addition, the approach described herein may be used *in vivo* to arrest or ameliorate rejection of previously engrafted tissue. In this regard, the composition may be prepared with pharmaceutically acceptable suitable carriers or diluents for injection or other means appropriate to the carrier. Generally, the recombinant virus carrying the vector construct is purified to a concentration ranging from 0.25% to 25%, and preferably about 5% to 20%, before formulation. Subsequently, after preparation of the composition, the recombinant vector will constitute about 10 ng to 1 μg of material per dose, with about 10 times this amount of material present as copurified contaminants. Preferably, the composition is prepared in 0.1-1.0 ml of aqueous solution formulated as described below.

Pharmaceutically acceptable carriers or diluents are those which are nontoxic to recipients at the dosages and concentrations employed. Representative examples of carriers or diluents for injectable solutions include water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or Tris-buffered saline) and containing one or more of mannitol, lactose, trehalose, dextrose, glycerol and ethanol, as well as polypeptides or proteins such as human serum albumin (HSA). One suitable composition comprises a recombinant virus carrying a vector construct in 10 mg/ml mannitol, 1 mg/ml HSA, 20mM Tris pH=7.2 and 150mM NaCl. In this case, since the recombinant virus carrying the vector construct represents approximately 10 ng to 1 μg of material, it may be less than 1% of the total high molecular weight material, and less than 1/100,000 of the total material (including water). This composition is generally stable at -70°C for at least six months. It will be evident that substantially equivalent dosages of the multivalent recombinant vector construct may be prepared. In this regard, the vector construct will constitute 100 ng to 100 ug of material per dose, with about 10 times this amount of material present as copurified contaminants. For recombinant viruses carrying the vector construct, the individual doses normally used are 10^6 to 10^{10} c.f.u. (e.g., colony forming units of neomycin resistance titered on HT1080 cells). These compositions are administered at one- to four-week intervals for three or four doses (at least initially). Subsequent booster shots may be given as one or two doses after 6-12 months, and thereafter annually.

The following examples are offered by way of illustration and not by way of limitation.

(TRANSPLANTATION - GRAFT REJECTION)

EXAMPLES

Example 1

PREPARATION OF MURINE RETROVIRAL PROVECTOR DNA

A. PREPARATION OF RETROVIRAL BACKBONE KT-3B

The Moloney murine leukemia virus (MoMLV) 5' long terminal repeat (LTR) EcoR I-EcoR I fragment, including *gag* sequences, from N2 vector (Armentano et al., J. Vir. 61:1647, 1987, Eglitis et al., Science 230:1395, 1985) in pUC31 plasmid is ligated into the plasmid SK^+ (Stratagene, San Diego, CA). The resulting construct is called N2R5. The N2R5 construct is mutated by site-directed *in vitro* mutagenesis to change the ATG start codon to ATT preventing *gag* expression. This mutagenized fragment is 200 base pairs (bp) in length and flanked by Pst I restriction sites. The Pst I-Pst I mutated fragment is purified from the SK^+ plasmid and inserted into the Pst I site of N2 MoMLV 5' LTR in plasmid pUC31 to replace the non-mutated 200 bp fragment. The plasmid pUC31 is derived from pUC19 (Stratagene, San Diego, CA) in which additional restriction sites Xho I, Bgl II, BssH II and Nco I are inserted between the EcoR I and Sac I sites of the polylinker. This construct is called $pUC31/N2R5g^M$.

The 1.0 kilobase (Kb) MoMLV 3' LTR EcoR I-EcoR I fragment from N2 was cloned into plasmid SK^+ resulting in a construct called $N2R3^-$. A 1.0 Kb Cla I-Hind III fragment is purified from this construct.

The Cla I-Cla I dominant selectable marker gene fragment from pAFVXM retroviral vector (Kriegler et al., Cell 38:483, 1984, St. Louis et al., PNAS 85:3150, 1988), comprising a SV40 early promoter driving expression of the neomycin phosphotransferase gene, is cloned into plasmid SK^+. A 1.3 Kb Cla I-BstB I gene fragment is purified from the SK^+ plasmid. This fragment, with the 3' LTR Cla I-Hind III fragment and the 5' LTR in $pUC31/N2R5g^M$ make up the KT-3B backbone.

An alternative selectable marker, phleomycin resistance (Mulsant et al., Som. Cell and Mol. Gen., 14:243, 1988, available from Cayla, Cedex, FR) may be used to make the retroviral backbone KT-3C, for use in transforming genes to cells that are already neomycin resistant. The plasmid pUT507 (Mulsant et al., Som. Cell and Mol. Gen., 14:243, 1988, available from Cayla, Cedex, FR) is digested with Nde I and the

ends blunted with Klenow polymerase I. The sample is then further digested with Hpa I, Cla I linkers ligated to the mix of fragments and the sample further digested with Cla I. The excess Cla I linkers are removed by digestion with Cla I and the 1.2 Kb Cla I fragment carrying the RSV LTR and the phleomycin resistance gene isolated by agarose gel electrophoresis followed by purification using Gene Clean (Bio101, San Diego, CA). This fragment is used in place of the 1.3 Kb Cla I-BstB I neomycin resistance fragment to give the backbone KT-3C.

The expression vector is constructed by a three part ligation in which the Xho I-Cla I fragment containing the gene of interest and the 1.0 Kb MoMLV 3' LTR Cla I-Hind III fragment are inserted into the Xho I-Hind III site of pUC31/N2R5g^M plasmid. The 1.3 Kb Cla I-BstB I neo gene, or 1.2 Kb Cla I phleomycin, fragment is then inserted into the Cla I site of this plasmid in the sense orientation.

Example 2

A. CLONING OF E3/19K GENE INTO KT-3B

i. ISOLATION AND PURIFICATION OF ADENOVIRUS

The isolation and purification of adenovirus is described by Green et al., Methods in Enzymology 58: 425, 1979. Specifically, five liters of Hela cells (3-6 x 10^5 cells/ml) are infected with 100-500 plaque forming units (pfu) per ml of adenovirus type 2 (Ad2) virions (ATCC VR-846). After incubation at 37°C for 30-40 hours, the cells are placed on ice, harvested by centrifugation at 230g for 20 minutes at 4°C, and resuspended in Tris-HCl buffer (pH 8.1). The pellets are mechanically disrupted by sonication and homogenized in trichlorotrifluoroethane prior to centrifugation at 1,000g for 10 min. The upper aqueous layer is removed and layered over 10 mls of CsCl (1.43 g/cm^3) and centrifuged in a SW27 rotor for 1 hour at 20,000 rpm. The opalescent viral band is removed and adjusted to 1.34 g/cm^3 with CsCl and further centrifuged in a Ti 50 rotor for 16-20 hours at 30,000 rpm. The visible viral band in the middle of the gradient is removed and stored at 4°C until purification of adenoviral DNA.

ii. ISOLATION AND PURIFICATION OF ADENOVIRUS DNA

The adenovirus band is incubated with protease for 1 hour at 37°C to digest proteins. After centrifugation at 7,800g for 10 minutes at 4°C, the particles are

solubilized in 5% sodium dodecyl sulfate (SDS) at room temperature for 30 minutes before being extracted with equal volumes of phenol. The upper aqueous phase is removed, re-extracted with phenol, extracted three times with ether, and dialyzed in Tris buffer for 24 hours. The viral Ad2 DNA is precipitated in ethanol, washed in ethanol, and resuspended in Tris-EDTA buffer, pH 8.1. Approximately 0.5 mg of viral Ad2 DNA is isolated from virus produced in 1.0 liter of cells.

iii. ISOLATION OF E3/19K GENE

The viral Ad2 DNA is digested with EcoR I (New England Biolabs, Beverly, MA) and separated by electrophoresis on a 1% agarose gel. The resulting 2.7 Kb Ad2 EcoR I D fragments, located in the Ad2 coordinate region 75.9 to 83.4, containing the E3/19K gene (Herisse et al., Nucleic Acids Research 8:2173, 1980, Cladaras et al., Virology 140:28, 1985) are eluted by electrophoresis, phenol extracted, ethanol precipitated, and dissolved in Tris-EDTA (pH 8.1).

iv. CLONING OF E3/19K GENE INTO KT-3B

The E3/19K gene is cloned into the EcoR I site of PUC1813. PUC1813 is prepared as essentially described by Kay et al., Nucleic Acids Research 15:2778, 1987 and Gray et al., PNAS 80:5842, 1983). The E3/19K is retrieved by EcoR I digestion and the isolated fragment is cloned into the EcoR I site of phosphatase-treated pSP73 plasmid, (Promega. Madison, WI). This construct is designated SP-E3/19K. The orientation of the SP-E3/19K cDNA is verified by using appropriate restriction enzyme digestion and DNA sequencing. In the sense orientation, the 5' end of the cDNA is adjacent to the Xho I site of the pSP73 polylinker and the 3' end adjacent to the Cla I site. The Xho I-Cla I fragment containing the E3/19K cDNA in either sense or antisense orientation is retrieved from the SP-E3/19K construct and cloned into the Xho I-Cla I site of the KT-3B retroviral backbone. This construct is called KT-3B/E3/19K.

B. CLONING OF PCR AMPLIFIED E3/19K GENE INTO KT-3B

i. PCR AMPLIFICATION OF E3/19K GENE

The Ad2 DNA E3/19K gene, including the amino terminal signal sequence, followed by the intraluminal domain and carboxy terminal cytoplasmic tail

which allow the E3/19K protein to embed itself in the endoplasmic reticulum (ER), is located between viral nucleotides 28,812 and 29,288. Isolation of the Ad2 E3/19K gene from the viral genomic DNA is accomplished by PCR amplification, with the primer pair shown below:

The forward primer corresponds to the Ad2 nucleotide sequences 28,812 to 28,835. (Sequence ID No. _____)

5'-3': TATATCTCCAGATGAGGTACATGATTTTAGGCTTG

The reverse primer corresponds to the Ad2 nucleotide sequences 29,241 to 29,213. (Sequence ID No. _____)

5'-3': TATATATCGATTCAAGGCATTTTCTTTTCATCAATAAAAC

In addition to the Ad2 complementary sequences, both primers contain a five nucleotide "buffer sequence" at their 5' ends for efficient enzyme digestion of the PCT amplicon products. This sequence in the forward primer is followed by the Xho I recognition site and by the Cla I recognition site in the reverse primer. Thus, in the 5' to 3' direction, the E3/19K gene is flanked by Xho I and Cla I recognition sites. Amplification of the E3/19K gene from Ad2 DNA is accomplished with the following PCR cycle protocol:

Temperature°C	Time (min)	No. Cycles
94	2	1
94	0.5	
55	0.17	5
72	3.5	
94	0.5	30
70	3.5	
72	10	10

ii. LIGATION OF PCR AMPLIFIED E3/19K GENE INTO KT-3B

The E3/19K gene from the SK-E3/19K construct, approximately 780 bp in length, is removed and isolated by 1% agarose/TBE gel electrophoresis as described in Example 2Bi. The Xho I-Cla I E3/19K fragment is then ligated into the KT-3B retroviral backbone. This construct is designated KT-3B/E3/19K . It is amplified by transforming DH5α bacterial strain with the KT-3B/E3/19K construct. Specifically, the bacteria is transformed with 1-100 ng of ligation reaction mixture DNA. The transformed bacterial cells are plated on LB plates containing ampicillin. The plates are incubated overnight at 37°C, bacterial colonies are selected and DNA prepared from them. The DNA is digested with Xho I and Cla I. The expected endonuclease restriction cleavage fragment sizes for plasmids containing the E3/19K gene are 780 and 1300 bp.

C. CLONING OF SYNTHESIZED E3/19K GENE INTO KT-3B

i. SYNTHESIS OF E3/19K GENE DNA

Chemical synthesis of synthetic DNA has been previously described (Caruthers et al., Methods in Enzymology 211:3, 1992). Sequences which encode the E3/19K gene are synthesized by the phosphotriester method on an Applied Biosystems Inc. DNA synthesizer, model 392 (Foster City, CA) using the PCR primers as the 5' and 3' limits and keeping the same Xho I and Cla I on the ends. Short oligonucleotides of approximately 14-40 nucleotides in length are purified by polyacrylamide gel electrophoresis and ligated together to form the single-stranded DNA molecule (Ferretti et al., PNAS 83:599, 1986).

ii. SEQUENCING OF E3/19K GENE DNA

Fragments are cloned into the bacteriophage vectors M13mp18 and M13mp19 (GIBCP, Gaithersburg, MD) for amplification of the DNA. The nucleotide sequence of each fragment is determined by the dideoxy method using the single-stranded M13mp18 and M13mp19 recombinant phage DNA as templates and selected synthetic oligonucleotides as primers. This confirms the identity and said structural integrity of the gene.

iii LIGATION OF SYNTHESIZED E3/19K GENE INTO KT-3B

The E3/19K gene is ligated into the KT-3B or KT-3C vector as previously described in Example 2B ii.

Example 3

CLONING OF AN ANTISENSE SEQUENCE OF A CONSERVED REGION OF MHC INTO KT-3C

A. CONSTRUCTION OF KT3CneoαMHC

The cDNA clone of the MHC class I allele CW3 (Zemmour et al., Tissue Antigens 39:249, 1992) is used as a template in a PCR reaction for the amplification of specific sequences conserved across different human MHC haplotypes to be inserted of the KT-3B backbone vector, into the untranslated region of the neomycin resistance gene.

The MHC class I allele CW3 cDNA is amplified between nucleotide sequence 147 to 1,075 using the following primer pairs:

The forward primer corresponds to MHC CW3 cDNA nucleotide sequence 147 to 166: (Sequence ID No. _____)

5'-3': TATATGTCGACGGGCTACGTGGACGACACGC

The reverse primer corresponds to MHC CW3 cDNA nucleotide sequence 1,075 to 1,056:
(Sequence ID No. ____)

5'-3': TATATGTCGACCATCAGAGCCCTGGGCACTG

In addition to the MHC class I allele CW3 complementary sequences, both primers contain a five nucleotide "buffer sequence" at their 5' ends for efficient enzyme digestion of the PCR amplicon products. The buffer sequence is followed by the Hinc II recognition sequence in both primers. Generation of the MHC amplicon with the primers shown above is accomplished using the PCR protocol described in section 2Bi.. This protocol is modified by using Vent polymerase (New England Biolabs, Beverly, MA) and further modified to include 1 minute extension times instead of 3.5 minutes. The Vent polymerase generates amplicons with blunt ends. Alternatively, the forward and reverse primers may contain only the MHC CW3 complementary sequences.

The MHC CW3 cDNA 950 bp amplicon product digested is purified with Gene Clean (Bio101, San Diego, CA) and digested with Hinc II. The fragment,

938 bp, is isolated by 1% agarose/TBE gel electrophoresis and purified with Gene Clean.

The MHC CW3 cDNA 938 bp fragment is inserted in the 3' untranslated region of the neomycin resistance gene in the antisense orientation. Specifically, the Hinc II recognition sequence at nucleotide sequence number 676 of the pBluescript II SK^+ (pSK^+) (Stratagene, San Diego, CA) plasmid is removed by digestion with Hinc II and Kpn I. The Kpn I 3' end is blunted with T4 DNA polymerase and the blunt ends are ligated. This plasmid is designated as pSKdlHII. As described in Example 1A, the 1.3 Kb Cla I- Cla I dominant selectable marker gene fragment from pAFVXM retroviral vector is cloned into the Cla I site of pSKdlHII. This plasmid is designated as pSKdlHII/SVneo. The MHC CW3 cDNA 938 bp fragment is inserted in an antisense orientation into the Hinc II site of pSKdlHII/SVneo located in the 3' untranslated region of the neomycin resistance gene. Confirmation that the MHC CW1 cDNA 938 bp fragment is present in the neomycin gene in an antisense orientation is determined by restriction endonuclease digestion and sequence analysis. This clone is designated as pSKdlHII/SVneo/αMHC.

Construction of KT3B/SVneo/αMHC is accomplished by a three way ligation, in which the Cla I 2.2 Kb SVneoαMHC fragment, and the 1.0 Kb MoMLV 3' LTR Cla I-Hind III fragment from $N2R3^-$, are inserted between the Cla I and Hind III sites of $pUC31/N2R5g^M$ plasmid as described in Example 1.

B. CONSTRUCTION OF KT3C/SVneo/VARNA/αMHC

High level MHC CW3 antisense RNA expression is accomplished by insertion of this sequence downstream of the Ad2 VARNA1 promoter. The Ad2 VARNA promoter-MHC antisense cDNA is assembled as a RNA polymerase III (pol III) expression cassette then inserted into the KT-3B or C backbone. In this pol III expression cassette, the Ad2 VARNA1 promoter is followed by the antisense αMHC cDNA, which in turn is followed by the pol III consensus termination signal.

The double stranded -30/+70 Ad2 VARNA1 promoter is chemically synthesized (Railey et al., Mol. Cell. Biol. 8:1147, 1988) and includes Xho I and Bgl II sites at the 5' and 3' ends, respectively.

The VARNA1 promoter, forward strand:
(Sequence ID No. ____)
5'-3': TCGAGTCTAGACCGTGCAAAAGGAGAGCCTGTAAGCGGGCACTCTTCC
GTGGTCTGGTGGATAAATTCGCAAGGGTATCATGGCGGACGACCGGGGT
TCGAACCCCGGA

The VARNA1 promoter, reverse strand:
(Sequence ID No. ____)
5'-3': GATCTCCGGGGTTCGAACCCCGGTCGTCCGCCATGATACCCTTGCGAA
TTTATCCACCAGACCACGGAAGAGTGCCCGCTTACAGGCTCTCCTTTT
GCACGGTCTAGAC

In order to form the double stranded VARNA1 promoter with Xho I and Bgl II cohesive ends, equal amounts of the single strands are mixed together in 10 mM $MgCl_2$, heated at 95°C for 5 min then cooled slowly to room temperature to allow the strands to anneal.

The MHC class I allele CW3 fragment, nucleotide sequence 653 to 854, from the plasmid pSKd1HII/SVneo/αMHC is amplified using the following primer pair:

The forward primer corresponds to nucleotide sequence 653 to 680:

5'-3': TATATCCTAGGTCTCTGACCATGAGGCCACCCTGAGGTG

The reverse primer corresponds to nucleotide sequence 854 to 827:

5'-3': TATATAGATCTACATGGCACGTGTATCTCTGCTCTTCTC

In addition to the MHC class I allele CW3 complementary sequences, both primers contain a five nucleotide "buffer sequence" at their 5' ends for efficient enzyme digestion of the PCR amplicon products. The buffer sequence is followed by the Avr II recognition sequence in the forward primer and by the Bgl II recognition sequence in the reverse primer, which allows insertion in an antisense orientation, relative to the Ad2 VARNA1 promoter in the pol III expression cassette. Generation of the MHC amplicon with the primers discussed above is accomplished with the PCR

protocol described in Example 2Bi modified to include 0.5 minute extension times instead of 3.5 minutes.

The MHC CW3 cDNA 223 bp amplicon product is purified with Gene Clean (Bio101, San Diego, CA), then digested with AvrII and BglII, and isolated by 2% NuSeive-1% agarose/TBE gel electrophoresis. The 211 bp band is then excised from the gel and the DNA purified with Gene Clean.

The double stranded pol III consensus termination sequence is chemically synthesized (Geiduschek et al., Annu. Rev. Biochem. 57:873, 1988) and includes Avr II and Cla I sites at the 5' and 3' ends, respectively.

The pol III termination sequence, forward primer:
(Sequence ID No. ____)
5'-3': CTAGGGCGCTTTTTGCGCAT

The pol III termination sequence, reverse primer:
(Sequence ID No. ____)
5'-3': CGATGCGCAAAAAGCGCC

In order to form the double stranded pol III transcription termination sequence with Avr II and Cla I cohesive ends, equal amounts of the single strands are mixed together in 10 mM $MgCl_2$, heated at 95°C for 5 min then cooled slowly to room temperature to allow the strands to anneal.

The pol III expression cassette for antisense αMHC class I allele CW3 is assembled in a four way ligation in which the Xho I-Bgl II Ad2 VARNA1 promoter fragment, the Bgl II-Avr II αMHC CW3 fragment, and the Avr II-Cla I transcription termination fragment, are cloned into $pSKII^+$ between the Xho I and Cla I sites. This construct is designated pSK/VARNA/αMHC.

Construction of KT3B/SVneo/VARNA/αMHC is accomplished in a two step ligation. The first step is a three way ligation in which the Xho I-Cla I VARNA/αMHC fragment and the 1.0 Kb MoMLV 3' LTR Cla I-Hind III fragment from $N2R3^-$, are inserted between the Xho I and Hind III sites of $pUC31/N2R5g^M$ plasmid as described in Example 1. This construct is designated KT3B/VARNA/αMHC. In the second ligation step the 1.3 Kb Cla I-BstB I SVneo fragment into the Cla I site of KT3B/VARNA/αMHC. This construct is designated KT3B/SVneo/VARNA/αMHC.

Example 4

CLONING A RIBOZYME THAT WILL CLEAVE A CONSERVED REGION OF MHC CLASS I HEAVY CHAIN INTO KT-3B

A. CONSTRUCTION OF pSK/VARNA/MHCHRBZ

In order to efficiently inhibit expression of MHC class I in transduced cells, a hairpin ribozyme with target specificity for the MHC class I allele is inserted into the KT3B/SVneo vector. The ribozyme is expressed at high levels from the Ad2 VARNA1 promoter. The MHC hairpin ribozyme (HRBZ) is inserted into the pol III pSK/VARNA/αMHC expression cassette described in Example 3.

The HRBZ and the MHC class I allele CW3 have the homologous sequence shown below:

(Sequence ID No. ____)

5'-3': GATGAGTCTCTCATCG

The HRBZ is designed to cleave after the A residue in the AGTC hairpin substrate motif contained in the target sequence. Following cleavage, the HRBZ is recycled and able to hybridize to, and cleave, other MHC class I RNA molecule.

Double-stranded HRBZ as defined previously (Hampel et al., Nucleic Acids Research 18:299, 1990), containing a four base "tetraloop" 3 and an extended helix 4, with specificity for the MHC class I homologous sequence shown above, is chemically synthesized and includes Bgl II and Avr II sites at the 5' and 3' ends, respectively.

The MHC HRBZ, sense strand:

(Sequence ID No. ____)

5'-3': GATCTCGATGAGAAGAACATCACCAGAGAAACACACGGACT
TCGGTCCGTGGTATATTACCTGGTAC

The MHC HRBZ, antisense strand:

(Sequence ID No. ____)

5'-3': CTAGGTACCAGGTAATATACCACGGACCGAAGTCCGTGTGTT
TCTCTGGTGATGTTCTTCTCATCGA

In order to form the double stranded MHC class I specific HRBZ with Bgl II and Avr II cohesive ends, equal amounts of the single strands are mixed together in 10 mM $MgCl_2$, heated at 95°C for 5 min then cooled slowly to room temperature to allow the strands to anneal.

The pol III expression cassette for the MHC HRBZ is assembled by ligation of the chemically synthesized double stranded MHC class I specific HRBZ with Bgl II and Avr II cohesive ends into Bgl II and Avr II digested and CIAP treated pSK/VARNA/αMHC, in which the αMHC sequence has been removed from the pol III expression vector. This plasmid is designated pSK/VARNA/MHCHRBZ and contains the Ad2 VARNA1 promoter followed by the MHC HRBZ, which in turn is followed by the pol III consensus termination sequence. The pol III expression components is flanked by Xho I and Cla I recognition sites.

B. CONSTRUCTION OF KT3B/SVneo/VARNA/MHCHRBZ

Construction of KT3B/SVneo/VARNA/MHCHRBZ is accomplished in a two step ligation. The first step is a three way ligation in which the Xho I-Cla I VARNA/MHCHRBZ fragment and the 1.0 Kb MoMLV 3' LTR Cla I-Hind III fragment from $N2R3^-$, are inserted between the Xho I and Hind III sites of $pUC31/N2R5g^M$ plasmid described in Example 1. This construct is designated KT3B/VARNA/MHCHRBZ. In the second step, the 1.3 Kb Cla I-BstB I SVneo fragment is ligated into the Cla I site of KT3B/VARNA/MHCHRBZ. This construct is designated KT3B/SVneo/VARNA/MHCHRBZ.

Example 5

CLONING OF PSF1 ANTISENSE cDNA

A. CONSTRUCTION OF KT3C/SVneo/αPSF1

The cDNA clone of PSF1 (Spies et al., Nature 351:323, 1991; Spies et al., Nature 348:744, 1990) is used as a template in a PCR reaction for the amplification of specific sequences to be inserted into the KT-3B backbone vector, into the untranslated region of the neomycin resistant gene. The PSF1 cDNA is amplified between nucleotide sequence 91 to 1,124 using the following primer pairs:

The forward primer corresponds to nucleotide sequence 91 to 111:
(Sequence ID No. ____)

5'-3': TATATGTCGACGAGCCATGCGGCTCCCTGAC

The reverse primer corresponds to nucleotide sequence 1,124 to 1,105:
(Sequence ID No. ____)

5'-3': TATATGTCGACCGAACGGTCTGCAGCCCTCC

In addition to the PSF1 complementary sequences, both primers contain a five nucleotide "buffer sequence" at their 5' ends for efficient enzyme digestion of the PCR amplicon products. The buffer sequence is followed by the Hinc II recognition sequence in both primers. Generation of the PSF1 amplicon with the primers discussed above is accomplished with the PCR protocol described in Example 2Bi. This protocol is modified by using Vent polymerase (New England Biolabs, Beverly, MA) and further modified to include 1 minute extension times instead of 3.5 minutes. The Vent polymerase generates amplicons with blunt ends.

B. CONSTRUCTION OF KT3B/SVneo/VARNA/αPSF1

High level PSF1 antisense expression is accomplished by insertion of this sequence downstream of the Ad2 VARNA1 promoter. The Ad2 VARNA promoter-PSF1 antisense cDNA is first assembled as a pol III expression cassette then inserted into the KT-3B backbone. In this pol III expression cassette, the Ad2 VARNA1 promoter is followed by the antisense PSF1 cDNA, which in turn is followed by the pol III consensus termination signal.

The nucleotide sequence 91 to 309 of the PSF1 cDNA are amplified in a PCR reaction using the following primer pair:

The forward primer corresponds to nucleotide sequence 91 to 111:
(Sequence ID No. ____)

5'-3': TATATCCTAGGGAGCCATGCGGCTCCCTGAC

The reverse primer corresponds to nucleotide sequence 309 to 288:
(Sequence ID No. ____)

5'-3': TATATAGATCTCAGACAGAGCGGGAGCAGCAG

In addition to the PSF1 complementary sequences, both primers contain a five nucleotide "buffer sequence" at their 5' ends for efficient enzyme digestion of the PCR amplicon products. The buffer sequence is followed by the Avr II recognition sequence in the forward primer and by the Bgl II recognition sequence in the reverse primer, which allows insertion in an antisense orientation, relative to the Ad2 VARNA1 promoter in the RNA polymerase III expression cassette. Generation of the PSF1 amplicon with the primers described above is accomplished with the PCR protocol described in Example 2Bi modified to include 0.5 minutes extension times instead of 3.5 minutes.

The MHC CW3 cDNA 240 bp amplicon product is purified with Gene Clean (Bio101, San Diego, CA), then digested with Avr II and Bgl II, and isolated by 2% NuSeive-1% agarose/TBE gel electrophoresis. The 211 bp band is then excised from the gel and purified with Gene Clean.

Construction of KT3B/SVneo/VARNA/αPSF1 is accomplished in two step ligation. The first step is a three-way ligation in which the Xho I-Cla I VARNA/αPSF1 fragment and the 1.0 Kb MoMLV 3' LTR Cla I-Hind III fragment from N2R3$^-$, are inserted between the Xho I and Hind III sites of pUC31/N2R5gM plasmid as described in Example 1. This construct is designated as KT3B/VARNA/αPSF1. In the second ligation step, the 1.3 kb Cla I-BstB I SVneo fragment is ligated into the Cla I site of KT3B/VARNA/αPSF1. This construct is designated KT3B/SVneo/VARNA/αPSF1.

Example 6

CLONING A RIBOZYME THAT WILL CLEAVE A CONSERVED REGION OF PSF1 INTO KT-3B

A. CONSTRUCTION OF pSK/VARNA/PSF1HRBZ

In order to efficiently inhibit expression of PSF1 in transduced cells, a hairpin ribozyme with target specificity for the PSF1 RNA is inserted into the KT3B/SVneo vector. The ribozyme is expressed at high levels from the Ad2 VARNA1 promoter. The PSF1 hairpin ribozyme (HRBZ) is inserted into the pol III pSK/VARNA/αMHC expression cassette described in Example 3. The PSF1 HRBZ-pol III expression cassette is then inserted into the KT3B/SVneo backbone vector.

The HRBZ and the PSF1 RNA have the homologous sequence shown below:

(Sequence ID No. ___)

5'-3': GCTCTGTCTGGCCAC

The HRBZ is designed to cleave after the T residue in the TGTC hairpin substrate motif contained in the target sequence. Following cleavage, the HRBZ is recycled and able to hybridize to, and cleave, other PSF1 RNA molecule.

Double-stranded HRBZ as defined previously (Hampel et al., Nucleic Acids Research 18:299, 1990), containing a four base "tetraloop" 3 and an extended helix 4, with specificity for the PSF1 homologous sequence shown above, is chemically synthesized and includes Bgl II and Avr II sites at the 5' and 3' ends, respectively.

The PSF1 HRBZ, sense strand:

(Sequence ID No. ____)

5'-3': GATCTGTGGCCAGACAGAGCACCAGAGAAACACACGGACTTCGG
TCCGTGGTATATTACCTGGTAC

The PSF1 HRBZ, antisense strand:

(Sequence ID No. ____)

5'-3': CTAGGTACCAGGTAATATACCACGGACCGAAGTCCGTGTGTT
TCTCTGGTGCTCTGTCTGGCCACA

In order to form the double stranded PSF1 specific HRBZ with Bgl II and Avr II cohesive ends, equal amounts of the single strands are mixed together in 10 mM $MgCl_2$ heated at 95°C for 5 min then cooled slowly to room temperature to allow the strands to anneal.

The pol III expression cassette for the PSF1 HRBZ is assembled by ligation of the chemically synthesized double stranded PSF1 specific HRBZ with Bgl II and Avr II cohesive ends into Bgl II and Avr II digested and CIAP treated pSK/VARNA/αMHC, in which the αMHC sequence has been gel purified away from the pol III expression vector. This plasmid is designated pSK/VARNA/PSF1HRBZ and contains the Ad2 VARNA1 promoter followed by the PSF1 HRBZ, which in turn is followed by the pol III consensus termination sequence. The pol III expression component is flanked by Xho I and Cla I recognition sites.

B. CONSTRUCTION OF KT3B/SVneo/VARNA/PSF1HRBZ

Construction of KT3B/SVneo/VARNA/MHCHRBZ is accomplished in a two step ligation. The first step is a three way ligation in which the Xho I-Cla I VARNA/PSF1HRBZ fragment and the 1.0 Kb MoMLV 3' LTR Cla I-Hind III fragment from $N2R3^-$, are inserted between the Xho I and Hind III sites of $pUC31/N2R5g^M$ plasmid as described in Example 1. This construct is designated KT3B/VARNA/PSF1HRBZ. In the second ligation step, the 1.3 Kb Cla I-BstB I SVneo fragment is ligated into the Cla I site of KT3B/VARNA/PSF1HRBZ. This construct is designated KT3B/SVneo/VARNA/PSF1HRBZ.

Example 7

CONSTRUCTION OF THE MULTIVALENT RECOMBINANT RETROVIRAL VECTOR KT3B-E3/19K

A variation of the retroviral vector KT3B-E3/19K can also be constructed containing both the E3/19K sequences and anti-sense sequences specific for a conserved region between the three class I MHC alleles A2, CW3 and B27, Examples 2 and 3. This vector, known as KT3B-E3/19K/αMHC, is designed to incorporate the MHC class I anti-sense sequences at the 3' end of the E3/19K sequence which would be expressed as a chimeric molecule. The retroviral vector, KT3B-E3/19K/αMHC, can be constructed by ligating a Cla I digested PCR amplified product containing the MHC anti-sense sequences into the Cla I site of the KT3B-E3/19K vector. More specifically, the cDNA clone of the MHC class I allele CW3 (Zemmour et al., Tissue Antigens 39:249, 1992) is amplified by PCR between nucleotides 653 and 854 using the following primer pair:

The forward primer of αMHC is:
(Sequence ID No. _____)
5'-3': ATTATCGATTCTCTGACCATGAGGCCACCCTGAGGTG

The reverse primer of αMHC is:
(Sequence ID No. _____)
5'-3': ATTAATCGATACATGGCACGTGTATCTCTGCTCTTCTC

The primer pairs are flanked by Cla I restriction enzyme sites in order to insert an amplified Cla I digested product into the partially pre-digested KT3B-E3/19K vector in the anti-sense orientation. By placing the Cla I fragment in the reverse orientation the vector will express the negative anti-sense strand upon transcription.

Example 8

TRANSDUCTION OF PACKAGING CELL LINE DA WITH THE RECOMBINANT RETROVIRAL VECTOR KT3B-E3/19K

A. PLASMID DNA TRANSFECTION

293 2-3 cells (a cell line derived from 293 cells ATCC No. CRL 1573, WO 92/05266) 5 x 10^5 cells are seeded at approximately 50% confluence on a 6 cm tissue culture dish. The following day, the media is replaced with 4 ml fresh media 4 hours prior to transfection. A standard calcium phosphate-DNA coprecipitation is performed by mixing 10.0 µg of KT3B-E3/19K plasmid and 10.0 µg MLP G plasmid with a 2M $CaCl_2$ solution, adding a 1x Hepes buffered saline solution, pH 6.9, and incubating for 15 minutes at room temperature. The calcium phosphate-DNA coprecipitate is transferred to the 293 2-3 cells, which are then incubated overnight at 37°C, 5% CO_2. The following morning, the cells are rinsed three times in 1x PBS, pH 7.0. Fresh media is added to the cells, followed by overnight incubation at 37°C, 10% CO_2. The following day, the media is collected off the cells and passed through a 0.45 µ filter. This supernatant is used to transduce packaging and tumor cell lines. Transient vector supernatant for other vectors are generated in a similar fashion.

B. PACKAGING CELL LINE TRANSDUCTION

DA cells (an amphotropic cell line derived from D-17 cells ATCC No. 183, WO 92/05266) are seeded at 5 x 10^5 cells/10 cm dish. Approximately 0.5 ml of the freshly collected 293 2-3 supernatant (or supernatant that has been stored at -70° C) is added to the DA cells. The following day, G418 is added to these cells and a drug resistant pool is generated over a period of a week. This pool of cells is dilution cloned by adding 0.8 -1.0 cell per well of 96 well plates. Twenty-four clones are expanded to 24 well plates, then to 6 well plates, at which time cell supernatants are collected for titering. DA clones are selected for vector production and called DA-E3/19K. Vector

supernatants are collected from 10cm confluent plates of DA-E3/19K clones cultured in normal media containing polybrene or protamine sulfate. Alternatively, vector supernatant can be harvested from bioreactors or roller bottles, processed and purified further before use.

For those vectors without a drug resistance marker or with a marker already in the packaging cell line, selection of stably transduced clones must be performed by dilution cloning the DA transduced cells one to two days after transducing the cells with 293 2-3 generated supernatant. The dilution clones are then screened for the presence of E3/19K expression by using reverse transcription of messenger RNA, followed by amplification of the cDNA message by the polymerase chain reaction, a procedure known as the RT-PCR. A commercial kit for RT-PCR is available through Invitrogen Corp. (San Diego, CA). RT-PCR should be performed on clones which have been propagated for at least 10 days and approximately 50 to 100 clones will need to be screened in order to find a reasonable number of stably transformed clones. In order to perform RT-PCR, specific primers will be required for each message to be amplified. Primers designed to amplify a 401 bp product for E3/19K message screening are as follows:

Screening primers for E3/19K are:
(Sequence ID No. _____)
5'-3': ATGAGGTACATGATTTTAGGCTTG

(Sequence ID No. ____)
5'-3': TCAAGGCATTTTCTTTTCATCAATAAAAC

Example 9

DETECTION OF REPLICATION COMPETENT RETROVIRUSES

The extended S^+L^- assay determines whether replication competent, infectious virus is present in the supernatant of the cell line of interest. The assay is based on the empirical observation that infectious retroviruses generate foci on the indicator cell line $MiCl_1$ (ATCC CCL 64.1). The $MiCl_1$ cell line is derived from the Mv1Lu mink cell line (ATCC CCL 64) by transduction with Murine Sarcoma Virus (MSV). It is a non-producer, non-transformed, revertant clone containing a murine sarcoma provirus that forms sarcoma (S^+) indicating the presence of the MSV genome

but does not cause leukemia (L-) indicating the absence of replication competent virus. Infection of $MiCl_1$ cells with replication competent retrovirus "activates" the MSV genome to trigger "transformation" which results in foci formation.

Supernatant is removed from the cell line to be tested for presence of replication competent retrovirus and passed through a 0.45 μ filter to remove any cells. On day 1, Mv1Lu cells are seeded at 1 x 10^5 cells per well (one well per sample to be tested) of a 6 well plate in 2 ml DMEM, 10% FBS and 8 μg/ml polybrene. Mv1Lu cells are plated in the same manner for positive and negative controls on separate 6 well plates. The cells are incubated overnight at 37°C, 10% CO_2. On day 2, 1.0 ml of test supernatant is added to the Mv1Lu cells. The negative control plates are incubated with 1.0 ml of media. The positive control consists of three dilutions (200 focus forming units (ffu), 20 ffu and 2 ffu each in 1.0 ml media) of MA virus (Miller et al., Molec. and Cell Biol. 5:431, 1985) which is added to the cells in the positive control wells. The cells are incubated overnight. On day 3, the media is aspirated and 3.0 ml of fresh DMEM and 10% FBS is added to the cells. The cells are allowed to grow to confluency and are split 1:10 on day 6 and day 10, amplifying any replication competent retrovirus. On day 13, the media on the Mv1Lu cells is aspirated and 2.0 ml DMEM and 10% FBS is added to the cells. In addition, the $MiCl_1$ cells are seeded at 1 x 10^5 cells per well in 2.0 ml DMEM, 10% FBS and 8 μg/ml polybrene. On day 14, the supernatant from the Mv1Lu cells is transferred to the corresponding well of the $MiCl_1$ cells and incubated overnight at 37°C, 10% CO_2. On day 15, the media is aspirated and 3.0 ml of fresh DMEM and 10% FBS is added to the cells. On day 21, the cells are examined for focus formation (appearing as clustered, refractile cells that overgrow the monolayer and remain attached) on the monolayer of cells. The test article is determined to be contaminated with replication competent retrovirus if foci appear on the $MiCl_1$ cells.

Example 10

TRANSDUCTION OF CELL LINES WITH E3/19K RETROVIRAL VECTOR

The following adherent human and murine cell lines are seeded at 5 x 10^5 cells/10 cm dish with 4 μg/ml polybrene: HT 1080 (ATCC No. CCL 121), Hela (ATCC No. CCL 2), and BC10ME (Patek et al., Cell. Immuno. 72: 113, 1982, ATCC No. TIB 85). The following day, 1.0 ml of filtered supernatant from the DA E3/19K pool is added to each of the cell culture plates. The following day, 800 ug/ml

G418 is added to the media of all cell cultures. The cultures are maintained until selection is complete and sufficient cell numbers are generated to test for gene expression. The transduced cell lines are designated HT 1080-E3/19K, Hela-E3/19K and BC10ME-E3/19K, respectively.

EBV transformed cell lines (BLCL), and other suspension cell lines, are transduced by co-cultivation with irradiated producer cell line, DA-E3/19K. Specifically, irradiated (10,000 rads) producer line cells are plated at 5 x 10^5 cells/6 cm dish in growth media containing 4 µg/ml polybrene. After the cells have been allowed to attach for 2-24 hours, 10^6 suspension cells are added. After 2-3 days, the suspension cells are removed, pelleted by centrifugation, resuspended in growth media containing 1mg/ml G418, and seeded in 10 wells of a round bottom 96 well plate. The cultures were expanded to 24 well plates, then to T-25 flasks.

Example 11

EXPRESSION OF E3/19K IN THE RECOMBINANT RETROVIRAL VECTOR CONSTRUCT KT3B-E3/19K

A. WESTERN BLOT ANALYSIS FOR E3/19K

Radio-immuno precipitation assay (RIPA) lysates are made from selected cultures for analysis of E3/19K expression. RIPA lysates are prepared from confluent plates of cells. Specifically, the media is first aspirated off the cells. Depending upon the size of the culture plate containing the cells, a volume of 100 to 500 µl ice cold RIPA lysis buffer (10 mM Tris, pH 7.4; 1% Nonidet P40 (Calbiochem, San Diego, CA); 0.1% SDS; 150 mM NaCl) is added to the cells. Cells are removed from plates using a micropipet and the mixture is transferred to a microfuge tube. The tube is centrifuged for 5 minutes to precipitate cellular debris and the supernatant is transferred to another tube. The supernatants are electrophoresed on a 10% SDS-PAGE gel and the protein bands are transferred to an Immobilon membrane in CAPS buffer (Aldrich, Milwaukee, WI) (10 mM CAPS, pH 11.0; 10% methanol) at 10 to 60 volts for 2 to 18 hours. The membrane is transferred from the CAPS buffer to 5% Blotto (5% nonfat dry milk; 50 mM Tris, pH 7.4; 150 mM NaCl; 0.02% sodium azide, and 0.05% Tween 20) and probed with a mouse monoclonal antibody to E3/19K (Severinsson et al., J. Cell. Biol. 101:540-547, 1985). Antibody binding to the membrane is detected by the use of ^{125}I-Protein A.

Example 12

FACS ANALYSIS OF E3/19K-VECTOR TRANSDUCED CELLS TO DEMONSTRATE DECREASED LEVELS OF CLASS I EXPRESSION COMPARED TO NON-TRANSDUCED CELLS.

Cell lines transduced with the E3/19K-vector are examined for MHC class I molecule expression by FACS analysis. Non-transduced cells are also analyzed for MHC class I molecule expression and compared with E3/19K transduced cells to determine the effect of transduction on MHC class I molecule expression.

Murine cell lines, BC10ME, BC10ME-E3/19K, P815 (ATCC No. TIB 64), and P815-E3/19K, are tested for expression of the H-2D^d molecule on the cell surface. Cells grown to subconfluent density are removed from culture dishes by treatment with Versene and washed two times with cold (4°C) PBS plus 1% BSA and 0.02% Na-azide (wash buffer) by centrifugation at 200g. Two million cells are placed in microfuge tubes and pelleted in a microfuge at 200g before removing the supernatant. Cell pellets are resuspended with the H-2D^d-specific Mab 34-2-12s (50µl of a 1:100 dilution of purified antibody, ATCC No. HB 87) and incubated for 30 min at 4°C with occasional mixing. Antibody labeled cells are washed two times with 1 ml of wash buffer (4°C) prior to removing the supernatant. Cells are resuspended with a biotinylated goat anti-mouse kappa light chain Mab (50µl, of a 1:100 dilution of purified antibody) (Amersham, Arlington Height, IL) and incubated for 30 min at 4°C. Cells are washed, resuspended with 50µl of avidin conjugated FITC (Pierce, Rockford, IL), and incubated for 30 min at 4°C. The cells are washed once more, resuspended in 1 ml of wash buffer, and held on ice prior to analysis on a FACStar Analyzer (Becton Dickinson, Los Angeles, CA). The mean fluorescence intensity of transduced cells is compared with that of non-transduced cells to determine the effect E3/19K protein has on surface M class I molecule expression.

Example 13

MURINE ALLOGENEIC CTL ASSAYS

H-2^d tumor cells (P815 or BC/10ME) irradiated with 10,000 rads are cultured with splenocytes isolated from six to eight week old female C57BL/6 (H-2^b)

mice (Harlan Sprague-Dawley, Indianapolis, IN) inducing allogeneic CTL. Specifically, 3 x 10^6 splenocytes/ml are cultured *in vitro* with 1.5-6.0 x 10^4 irradiated tumor cells/ml for 4-5 days at 37°C in T-25 flasks. Culture medium consists of RPMI 1640; 5% FBS, heat-inactivated; 1 mM pyruvate; 50 µg/ml gentamicin and 10^{-5} M 2-mercaptoethanol. Effector cells are harvested 5 days later and tested using various effector:target cell ratios in 96 well microtiter plates in a standard 4-6 hour assay. The assay employs $Na_2{}^{51}CrO_4$-labeled, 100 µCi, 1 hr at 37°C, (Amersham, Arlington Heights, IL) target cells at 4-10 x 10^3 cells/well with the final total volume per well of 200 µl. Following 4-6 hour incubation at 37°C, 100 µl of culture medium is removed and analyzed in a WALLAC gamma spectrometer (Gaithersburg, MD). Spontaneous release (SR) is determined as CPM from targets plus medium and maximum release (MR) is determined as counts per minute (CPM) from targets plus 1M HCl. Percent target cell lysis is calculated as: [[(effector cell + target CPM) - (SR)]/[(MR) - (SR)]] x 100. Spontaneous release values of targets are typically 10%-20% of the MR. Tumor cells that have been transduced with the gene of interest (ribozyme, E3/19K, antisense, etc.) are used as stimulator and/or target cells in this assay to demonstrate the reduction of allogeneic CTL induction and detection.

Example 14

FACS ANALYSIS OF E3/19K-VECTOR TRANSDUCED HUMAN CELLS TO DEMONSTRATE DECREASED LEVELS OF MHC CLASS I EXPRESSION COMPARED TO NON-TRANSDUCED CELLS

Cell lines transduced with the E3/19K vector are examined for class I molecule expression by FACS analysis. Non-transduced cells are also be analyzed for class I molecule expression to compare with E3/19K transduced cells and to determine the effect that transduction has on class I molecule expression.

Two human cell lines JY-E3/19K and JY (ATCC No. ____) are used to test for expression of the HLA-A2 molecule on the cell surface. Suspension cells grown to 10^6 cells/ml are removed from culture flasks by pipet and washed two times with cold (4°C) PBS plus 1% BSA and 0.02% Na-azide (wash buffer) by centrifugation at 200g. Two million (2 x 10^6) cells are placed in microfuge tubes, pelleted in at 200g, and the supernatant is removed. Cell pellets are resuspended with the HLA-A2-specific Mab BB7.2 (50µl of a 1:100 dilution of purified antibody, ATCC No. HB 82) and incubated with antibody for 30 min at 4°C with occasional mixing. Antibody labeled

cells are washed two times with 1 ml of wash buffer (4°C). Prior to removing the supernatant, the cells are resuspended with a biotinylated rat anti-mouse kappa light chain Mab (50µl, of a 1:100 dilution of purified antibody) and incubated for 30 min at 4°C. Cells are washed, resuspended with 50µl of avidin conjugated FITC (Pierce, Rockford, IL), and incubated for 30 min at 4°C. The cells are washed once more, and resuspended in 1 ml of wash buffer, and held on ice prior to analysis on a FACStar Analyzer. The mean fluorescence intensity of transduced cells is compared with that of non-transduced cells to determine the effect E3/19K protein has on surface MHC class I molecule expression.

Example 15

MEASUREMENT OF THE IMMUNE RESPONSE TO E3/19K-TRANSDUCED AND NONTRANSDUCED EBV-TRANSFORMED HUMAN JY CELLS BY ALLOGENEIC HUMAN CTL LINES

Human CTL lines can be propagated from donor blood samples using allogeneic EBV-transformed cell lines as stimulators. These CTL lines are propagated with JY cells which possess the A2 molecule and can lyse JY target cells. A chromium release assay can be performed with these CTL lines and JY target cells that have been transformed with the E3/19K gene or nontransformed JY target cells. The E3/19K transformed JY target cells are used to demonstrate decreased recognition and lysis of this cell when compared to nontransformed JY target cells. These results indicate that cell transformation with agents that decrease MHC class I surface expression also decreases MHC class I restricted cell mediated immune responses in an *in vitro* human cell model system.

An allogeneic CTL reaction is induced by culturing 10^6 irradiated (10,000 rad) JY cells with 10^7 PBMC from a non-HLA-A2 person in 10 mls of culture medium at 37°C 5% CO_2 for 7-10 days. The culture medium consists of RPMI 1640 supplemented with 5% heat inactivated fetal bovine serum preselected for CTL growth, 1 mM sodium pyruvate and nonessential amino acids. After the 7-10 day incubation the effector cells are harvested and tested in a standard 4-6 hour chromium release assay using ^{51}Cr labeled JY cells as the positive control and ^{51}Cr labeled JY-E3/19K. JY and JY-E3/19K cells are labeled with 300 µCi of $Na_2{}^{51}CrO_4$ for 1 hour at 37°C, then washed, counted, and used in the assay at 4×10^3 cells/well with the final total volume per well of 200 µl. Following incubation, 100 µl of culture medium is removed and

analyzed in a WALLAC gamma spectrometer (Gaithersburg, MD). Spontaneous release (SR) is determined as counts per minute (CPM) from targets plus medium and maximum release (MR) is determined as CPM from targets plus 1M HCl. Percent target cell lysis is calculated as: [[(effector cell + target CPM) - (SR)]/[(MR) - (SR)]] x 100. Spontaneous release values of targets are typically 10%-30% of the MR. Tumor cells that have been transduced with the gene of interest (ribozyme, E3/19K, antisense, etc.) are used as stimulator and/or target cells in this assay to demonstrate the reduction of allogeneic CTL induction and detection as compared to the non-transduced line which is the positive control.

Example 16

ALLOGENEIC MARROW GRAFTS

i. REMOVE 4L OF BONE MARROW FROM C3H (H-2^k) AND BALB/C (H-2^d)

Mouse femurs are dissected and exposed. The bone marrow plugs are removed using a number 23 gauge needle and syringe. The marrow is collected and resuspended marrow in Hank's balanced salt solution (Mauch et al., PNAS 77:2927, 1980)

ii. TRANSDUCTION OF MARROW CELLS WITH E19 RETROVIRAL VECTOR

Marrow cells are prepared by centrifugation and resuspension in 1.0 ml DMEM and 10% FBS containing E3/19K vector. The marrow cells and E3/19K retroviral vector is incubated for 4 hours at 33°C then 9 mls of Fischer's medium supplemental with 25% donor horse serum and 0.1 mM hydrocortisone sodium succinate. After 24 hours the marrow cells were washed and resuspended in HBSS at 2 x 10^6 cells/ ml for injection.

iii. INJECTION OF MARROW CELLS INTO MICE

The C57BL/6 (B6, H-2^b) mice are irradiated with 700 rads of gamma irradiation just prior to injection. Two groups of B6 mice are injected intravenously

with 0.5 ml of C_3H marrow cells. After 5 days the mice are again irradiated with 700 rads and injected intravenously with 0.5 ml of either vector-transduced C_3H marrow cells or untreated C_3H marrow cells. Lethally irradiated naive B6, mice are injected intravenously with 0.5ml (1×10^6) of C_3H bone marrow cells for the positive control and 0.5ml (1×10^6) of Balb/c bone marrow cells for the negative control.

iv. EVALUATION OF GRAFT REJECTIONS

The bone marrow graft rejections are evaluated 5 days following injection by either of the two methods:

a. After sacrificing the mice, the spleens are removed and placed into 10% formalin. Spleen colonies are counted and recorded.

b. Mice are injected with FUdR (Sigma, St. Louis, MO) and 30 minutes later with ^{125}I-IUdR (Amersham, Arlington Height, IL). After 18 hours of incubation, the spleens are removed and ^{125}I-IUdR incorporation determined in the spleens of with replicating bone marrow cells.

c. The value of incorporated radioactivity determined in the syngeneic growth control is arbitrarily set at 100 U, and all values in the experimental groups are normalized relative to this control. Animals with ~10 U show no visible spleen colonies, whereas animals with 50 to 100 U have greater than 200 spleen colonies. Animals that show less than U are considered to express strong rejection, those with 10 to 30 U are considered to express weak rejection, and those with greater than 30 U show no significant rejection.

RECIPIENT	1°	2°	RESULT (MARROW GROWTH)
B6 (H-2^b)		Balb/c (H-2^d)	-
B6 (H-2^b)		C_3H (H-2^k)	+
B6 (H-2^b)	C_3H	C_3H	-
B6 (H-2^b)	C_3H	C_3H-E3/19K	+

Example 17

A. ISOLATION AND TRANSDUCTION OF BONE MARROW CELLS

Pluripotent hematopoeitic stem cells, $CD34^+$ are collected from the bone marrow of a patient by a syringe evacuation performed by known techniques. Alternatively, $CD34^+$ cells may also be obtained from the cord blood of an infant if the patient is diagnosed before birth. Generally, 20 bone-marrow aspirations are obtained by puncturing femoral shafts or from the posterior iliac crest under local or general anesthesia. Bone marrow aspirations are then pooled and suspended in Hepes-buffered Hanks' balanced salt solution containing heparin sulfate at 100 Units/ml and deoxyribonuclease I at 100 μg/ml and then subjected to a Ficoll gradient separation. The buffy coated marrow cells are then collected and washed according to CEPRATE™ LC (CD34) Separation system (Cellpro, Bothell, WA). The washed buffy coated cells are then stained sequentially with anti-CD34 monoclonal antibody, washed, then stained with biotinylated secondary antibody supplied with the CEPRATE™ system. The cell mixture is then loaded onto the CEPRATE™ avidin column. The biotin-labeled cells are adsorbed onto the column while unlabeled cells pass through. The column is then rinsed according to the CEPRATE™ system directions and $CD34^+$ cells eluted by agitation of the column by manually squeezing the gel bed. Once the $CD34^+$ cells are purified, the purified stem cells are counted and plated at a concentration of 1×10^5 cells/ml in Iscove's modified Dulbecco's medium, IMDM (Irvine Scientific, Santa Ana, CA), containing 20% pooled non-heat inactivated human AB serum (hAB serum).

After purification of $CD34^+$ cells, several methods of transducing purified stem cells may be performed. One approach involves transduction of the purified stem cell population with vector containing supernatant cultures derived from vector producing cells. A second approach involves co-cultivation of an irradiated monolayer of vector producing cells with the purified population of non-adherent $CD34^+$ cells. A third and preferred approach involves a similar co-cultivation approach, however the purified $CD34^+$ cells are pre-stimulated with various cytokines and cultured 48 hours prior to the co-cultivation with the irradiated vector producing cells. Pre-stimulation prior to transduction increases effective gene transfer (Nolta et al., Exp. Hematol. 20:1065; 1992). The increased level of transduction is attributed to increased proliferation of the stem cells necessary for efficient retroviral

transduction. Stimulation of these cultures to proliferate also provides increased cell populations for re-infusion into the patient.

Pre-stimulation of the $CD34^+$ cells is performed by incubating the cells with a combination of cytokines and growth factors which include IL-1, IL-3, IL-6 and mast cell growth factor (MGF). Pre-stimulation is performed by culturing 1-2 x 10^5 $CD34^+$ cells / ml of medium in T25 tissue culture flasks containing bone marrow stimulation medium for 48 hours. The bone marrow stimulation medium consists of IMDM containing 30% non-heat inactivated hAB serum, 2mM L-glutamine, 0.1 mM 2-mercaptoethanol, 1μM hydrocortisone, and 1% deionized bovine serum albumin. All reagents used in the bone marrow cultures should be screened for their ability to support maximal numbers of granulocyte erythrocyte macrophage megakaryocyte colony-forming units from normal marrow. Purified recombinant human cytokines and growth factors (Immunex Corp., Seattle, WA) for pre-stimulation should be used at the following concentrations: *E. coli*-derived IL-1α (100 U/ml), yeast-derived IL-3 (5 ng/ml), IL-6 (50 U/ml), and MGF (50 ng/ml) [Anderson et al., Cell Growth Differ. 2:373, 1991].

After prestimulation of the $CD34^+$ cells, the cells are then transduced by co-cultivating on to the irradiated DA-based producer cell line (expressing the E3/19K vector) in the continued presence of the stimulation medium. The DA vector producing cell line is first trypsinized, irradiated using 10,000 rad and replated at 1-2 x 10^5/ml of bone marrow stimulation medium. The following day, 1-2 x 10^5 prestimulated $CD34^+$ cells /ml were added onto the DA vector producing cell line monolayer followed by polybrene (Sigma, St. Louis, MO) to a final concentration of 4ug/ml. Co-cultivation of the cells should be performed for 48 hours. After co-cultivation , the $CD34^+$ cells are collected from the adherent DA vector producing cell monolayer by vigorous flushing with medium and plated for 2 hours to allow adherence of any dislodged vector producing cells. The cells are then collected and expanded for an additional 72 hours. The cells are collected and frozen in liquid nitrogen using a cryo-protectant in aliquots of 1 x 10^7 cells per vial. Once the transformed $CD34^+$ cells have been tested for the presence of adventitious agents, frozen transformed $CD34^+$ cells may be thawed, plated to a concentration of 1 x 10^5 cells/ml and cultured for an additional 48 hours in bone marrow stimulation medium. Transformed cells are then collected, washed twice and resuspended in normal saline. The number of transduced cells used to infuse back into the patient per infusion is projected to be at a minimum of 10^7 x 10^8 cells per patient per injection. The site of infusion may be directly into the patients bone marrow or i.v. into the peripheral blood stream.

B. ISOLATION OF PANCREATIC ISLET CELLS

Procedures for the isolation of human pancreatic islet cells have been previously described (Warnock et al., Diabetologia 35:85 1992; Warnock et al., Transplantation 45:957, 1988). The pancreas is obtained from adult human cadaver organ donors at the National Disease Research Interchange in Philadelphia, PA. It is removed by laparotomy by dividing the gastrocolic omentum and splenic ligaments. The neck of the pancreas is freed from the portal vein and the remainder of the gland is detached from the retroperitoneum. The pancreas is weighed and immersed into 4°C Hanks' balanced salt solution (HBSS). The pancreatic duct at the head is cannulated with a 16 gauge cannula and then HBSS-containing collagenase type XI (Sigma Chemicals, St. Louis, MO) is injected. Upon transfer to a cooling tray, the pancreatic duct is exposed at the middle of the gland and two additional 16 gauge cannulas are inserted into this portion of the duct. Each pancreatic duct is perfused with a collagenase solution at 4°C and then gradually warmed to 38°C. Digestion of the pancreas is judged complete when the islets dissociate freely from the exocrine tissue as determined microscopically. The digested tissue is transferred to HBSS containing 2% (v/v) newborn calf serum (Gibco, Burlington, Ontario, Canada) at 4°C and gently teased apart. The tissue is washed, passed through needles of progressively smaller sizes and suspended in tissue culture medium 199 (Gibco, Burlington, Ontario, Canada) at 4°C using 0.6 g of tissue per 3.4 ml of medium. Aliquots of tissue suspension are mixed with media and Ficoll (Density 1.125, Sigma, St. Louis, MO) and centrifuged in a discontinuous Ficoll gradient at 550g for 25 minutes at 22°C. Interfaces are collected, washed, and resuspended in culture medium. The cells are then transformed in one of the several ways outlined in the specification. Since pancreatic cells do not replicate efficiently in culture it may be useful to transform with DNA or vector systems capable of infecting non-replicating cells, for example sindbis virus or adeno-associated virus. The genes introduced are those described for the retroviral vector system.

Example 18

REPLACEMENT OF TRANSPLANTABLE PANCREATIC ISLET CELLS

Replacement of pancreatic islet cells can be accomplished by using the epiploic flap method as previously described (Altman et al., Hormone and Metabolic

Res. Suppl. 25:136, 1990). After transduction of islets as described above, cells are pelleted and resuspended in 10 mls of a heparinized solution of HBSS. The vascular circle of the greater curve tied to the epiploon was cut in its middle part, released from the stomach and mobilized with its epiploic flap. A retrograde injection of the cell solution was embolized into the right extremity of the gastro-epiploic artery. This evenly distributed the islet preparation into the epiploic flap which was set subcutaneously in the paraumbilical area.

Islet encapsulation, or the development of a bioartificial pancreas can also be used. Microencapsulation using an arginate poly-L-lysine membrane has been demonstrated by several groups (Fritschy et al., Diabetes 40:37, 1991; Krestow et al., Transplantation 51:651, 1991; Mazaheri et al., Transplantation 51:750, 1991) This technique is applicable to both xenogeneic and allogeneic islets and can sustain prolonged normoglycemia.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

1. Tissue cells of an animal transformed with a recombinant vector construct which directs the expression of a protein or active portion of a protein capable of inhibiting MHC antigen presentation, for use in a method of suppressing graft rejection.

2. The cells of claim 1 wherein the protein is capable of binding β_2-microglobulin.

3. The cells of claim 1 wherein the protein is capable of binding the MHC class I heavy chain molecule intracellularly.

4. The cells of claim 1 wherein the protein is E3/19K or H301.

5. Tissue cells transformed with a recombinant vector construct which transcribes an antisense message, the antisense message capable of inhibiting MHC antigen presentation, for use in a method of suppressing graft rejection.

6. The cells of claim 5 wherein said recombinant vector construct transcribes an antisense message which binds to a conserved region of MHC class I heavy chain transcripts.

7. The cells of claim 5 wherein said recombinant vector construct transcribes an antisense message which binds the β_2-microglobulin transcript.

8. The cells of claim 5 wherein said recombinant vector construct transcribes an antisense message which binds the PSF1 transporter protein transcript.

9. Tissue cells transformed with a recombinant vector construct which transcribes a ribozyme, said ribozyme capable of inhibiting MHC antigen presentation, for use in a method of suppressing graft rejection.

10. The cells of claim 9 wherein said recombinant vector construct transcribes a ribozyme that cleaves a conserved region of MHC class I heavy chain transcripts.

11. The cells of claim 9 wherein said recombinant vector construct transcribes a ribozyme that cleaves the β_2-microglobulin transcript.

12. The cells of claim 9 wherein said recombinant vector construct transcribes a ribozyme that cleaves the PSF1 transporter protein transcript.

13. The cells of any one of claims 1, 5 or 9 wherein said recombinant vector construct is carried by a recombinant virus selected from the group consisting of togaviridae, picornaviridae, poxviridae, adenoviridae, parvoviridae, herpesviridae and paramyxoviridae viruses.

14. The cells of any one of claims 1, 5 or 9 wherein said recombinant vector construct is carried by a recombinant virus selected from the group consisting of poliovirus, rhinovirus, vaccinia virus, influenza virus, adenovirus, adeno-associated virus, herpes simplex virus and measles virus.

15. The cells of any one of claims 1, 5 or 9 wherein said recombinant vector construct is carried by coronavirus.

16. The cells of any one of claims 1, 5 or 9 wherein said recombinant vector construct is carried by Sindbis virus.

17. The cells of any one of claims 1, 5 or 9 wherein said recombinant vector construct is a recombinant viral vector construct.

18. The cells of any one of claims 1, 5 or 9 wherein said recombinant vector construct is a recombinant retroviral vector construct.

19. The cells of any one of claims 1, 5 or 9 wherein said tissue cells are transformed *ex vivo* with the recombinant vector construct .

20. Tissue cells transformed with a multivalent recombinant vector construct which directs the expression of a protein or active portion of a protein capable of inhibiting MHC antigen presentation, and an antisense or ribozyme capable of inhibiting MHC antigen presentation, for use in a method of suppressing graft rejection.

21. Tissue cells transformed with a multivalent recombinant vector construct which directs the expression of an antisense message and ribozyme capable of inhibiting MHC antigen presentation, for use in a method of suppressing graft rejection.

22. Tissue cells transformed with a multivalent recombinant vector construct which directs the expression two or more proteins or active portions of said proteins capable of inhibiting MHC antigen presentation, or two or more antisense messages capable of inhibiting MHC antigen presentation, or two or more ribozymes capable of inhibiting MHC antigen presentation, for use in a method of suppressing graft rejection.

23. The cells of claim 22 wherein said multivalent recombinant vector construct directs the expression of the E3/19K or H301 proteins or an active portion of the E3/19K or H301 proteins, and a second protein or active portion of said second protein selected from the group consisting of E3/19K and H301.

24. The cells of claim 20 or 21 wherein said multivalent recombinant vector construct transcribes an antisense message which binds to the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

25. The cells of claim 22 wherein said multivalent recombinant vector construct transcribes two antisense messages, the first transcribed antisense message binding to a conserved region of MHC class I heavy chain transcripts and the second transcribed antisense message binding to the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

26. The cells of claim 22 wherein said multivalent recombinant vector construct transcribes two antisense messages, the first transcribed antisense message binding to the β_2-microglobulin molecule and the second transcribed antisense message binding to the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

27. The cells of claim 22 wherein said multivalent recombinant vector construct transcribes two antisense messages, the first transcribed antisense message binding to the PSF1 molecule and the second transcribed antisense message binding to the transcript

of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

28. The cells of claim 20 or 21 wherein said multivalent recombinant vector construct transcribes a ribozyme that cleaves the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

29. The cells of claim 22 wherein said multivalent recombinant vector construct transcribes two ribozymes, the first transcribed ribozyme cleaving a conserved region of MHC class I heavy chains and the second transcribed ribozyme cleaving the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

30. The cells of claim 22 wherein said multivalent recombinant vector construct transcribes two ribozymes, the first transcribed ribozyme cleaving the β_2-microglobulin molecule and the second transcribed ribozyme cleaving the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

31. The cells of claim 22 wherein said multivalent recombinant vector construct transcribes two ribozymes, the first transcribed ribozyme cleaving the PSF1 molecule and the second transcribed ribozyme cleaving the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, β_2-microglobulin and PSF1 transporter protein.

32. The cells of any one of claims 20, 21, or 22 wherein said multivalent recombinant vector construct is carried by a recombinant virus selected from the group consisting of togaviridae, picornaviridae, poxviridae, adenoviridae, parvoviridae, herpesviridae and paramyxoviridae viruses.

33. The cells of any one of claims 1, 5, 9, 20, 21, or 22 wherein the tissue cells are selected from the group consisting of bone marrow cells, pancreatic islet cells, fibroblast cells, corneal cells and skin cells.

34. A recombinant vector construct which directs the expression of E3/19K or H301.

35. A recombinant vector construct which transcribes an antisense message which binds the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, the β_2-microglobulin and the PSF1 transporter protein.

36. A recombinant vector construct which transcribes a ribozyme that cleaves the transcript of a protein selected from the group consisting of a conserved region of MHC class I heavy chains, the β_2-microglobulin and the PSF1 transporter protein.

37. The recombinant vector construct carried by a recombinant virus selected from the group consisting of poliovirus, rhinovirus, vaccinia virus, influenza virus, adenovirus, adeno-associated virus, herpes simplex virus and measles virus.

38. The recombinant vector construct carried by a recombinant virus selected from the group consisting of togaviridae, picornaviridae, poxviridae, adenoviridae, parvoviridae, herpesviridae, and paramyxoviridae.

39. The recombinant viral vector construct of any one of claims 34-38 wherein said vector construct is a recombinant viral vector construct.

40. The recombinant viral vector construct of any one of claims 34-38 wherein said vector construct is a recombinant retroviral vector construct.

41. A tissue cell transformed with a recombinant vector construct according to any one of claims 34-38.

42. A tissue cell transformed with a recombinant viral vector construct according to claim 39.

43. A tissue cell transformed with a recombinant retroviral vector construct according to claim 40.

44. A pharmaceutical composition comprising the transformed tissue cells of any one of claims 41-43 and a physiologically acceptable carrier or diluent.